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# Unifying Scaler for Bioassay Tests

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# A UNIFYING SCALER FOR BIOASSAY TESTS

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ABSTRACT

An extensive set of interlaboratory root bioassay data was unified using centroids of individual tests as scalers. It is shown that the dose response obeys a first order differential equation with the constant of the equation related to the sensitivity of the dose response relationship evaluated in the central portion of the unified curve. The solution to the equation approximates the data rather well, making it suitable for prediction. A wide range of applications of the results demonstrate the utility of the approach and its potential for use in other bioassay work.

Key words: Scaling, centroid, bioassay, root elongation.

## INTRODUCTION

Individual toxicological tests fluctuate widely in their dose-response relationships. These variations are quite common for the same organisms and the same chemicals within and between laboratories at an identical dose level. In addition, toxicant dose levels are widely spaced, reflecting broadly different tolerences of organisms to chemicals. As a result, it is difficult to detect a general trend relating dose to response in laboratory bioassays. The discovery of a universal relationship is useful for estimating the precision of an individual test, designing bioassay experiments and predicting chemical effects.

The likelihood, even the expectation, of discovering a truly universal dose-response relationship for all bioassay tests is remote. A more realistic objective is to synthesize existing data for the same or for similar classes of chemicals or organisms, expecting to establish a pattern that represents a collective knowledge. Thus, in place of a universal relationship, it is reasonable to seek a "unified relationship" that summarizes a group of tests. If this is done systematically so new understanding can be added to existing knowledge, the likelihood of discovering a more general relationship that approaches a universal law would be greater.

A unified relationship emerges in the limit as the number of experiments in the data base grows to a point where a stable dose-response pattern changes little with added new data. The individual data points of an experiment deviate from the unified curve to a varying degree due to uncontrollable factors. It is conceivable that bioassay experiments share generally common uncontrollable factors. To the extent that it might be so, it is desirable to isolate scaled biological response from variability due to these factors. If we consider the unified curve to represent a biological response of organisms

to varying chemical dose levels, the mean deviation of many individual data points along the dose-response curve would constitute the experimental error associated with a variety of uncontrolable factors.

There may not exist a theoretical justification to expect a unified system. Nevertheless, it is helpful to anticipate a feasible theory from the data, hence facilitating data use and error analysis. The development of a unified dose-response pattern and establishing experimental error patterns relative to it are the major objectives of this paper.

## SCALER CRITERIA

The discovery of a unified relationship from a set of data is dependent on the success in finding one or more scalers with the ideal properties: (1) The numerical value of the scalers can be easily calculated from the outcome of an individual experiment. (2) They summarize all the essential features of an experiment. (3) They relate objectives of an experiment to the causal factors underlying dose-response relationships. (4) The results based on using the scalers are easily understandable, believable, and can be translated to conventional EC50, LC50, etc. (5) Their use does not modify the nature of the data, i.e., the original data are not lost or obscured by truncation or excessive smoothing.

Scalers with ideal properties can be found for many physical systems that have been described by differential equations. The equations facilitate the use of theory of similarity, a general theory commonly applied to engineering problems to calculate the scalers that best unify experimental data. Extension of the theory to biological problems has been attempted by Riley et al. 1981; Shirazi, 1980; Shirazi and Riley, 1981; and Shirazi et al. 1984. With these systems, the general mathematical equations can be used in an inductive way to

calculate the scalers. When a general equation cannot be established, a pattern must be developed by means of multivariate statistical methods, a purely deductive approach, or from a mixed deductive-intuitive approach. Shirazi and Seim (1981) used the 'mixed' approach to unify a diverse data set that related the embryo survival of salmonids to the conditions of the spawning habitat in a stream. Their approach can be summarized in these steps: (1) Identify important physical and biological components of the problem. (2) Calculate measures based on these components to be used as tentative scalers of the problem. (3) Test the interaction between the physical and biological scalers using the data. Success is evidenced by the degree of unification achieved in a trial. This mixed approach will be used to analyze bioassay data.

So our approach may appear atypical to some readers that two or more readings of the paper may be required, lightly at first, in order to capture its full flavor.

## METHODS AND ANALYSIS

The scalers we propose to use are two coordinates of the centroid obtained directly from the test data. The first which we will refer to as DOSE PIVOT and designate by the symbol DP locates the fulcrum of a dose-response test on the dose axis. The second is, likewise, the RESPONSE PIVOT RP. These two numbers which in a manner identify a dose-response test will be used to scale or "standardize" the dose and response values of that test.

The procedure in using the scalers will be demonstrated by means of an example.

A data set exemplifying the diversity of bioassay testing problems is reported by Ratsch (1983). We begin by selecting several representative tests

(Table 1) from that set to demonstrate the application of our method. We then will apply the method to the entire data base and compare results obtained from it with the analysis of Ratsch (1983).

Table 1. Typical examples of root bioassay showing widely spaced dose levels and variability of response relative to laboratory organisms or chemicals.

| Dose | Wheat/2,4-D | | Wheat/Carbowax | | | Radish/2,4-D | |
|---|---|---|---|---|---|---|---|
| Lab No. | Lab 3 | Lab 6 | Lab 1 | | Lab 2 | Lab 4 | Lab 5 |
| Test No. | 519 | 536 | 543 | 544 | 548 | 931 | 939 |
| Dose (mg/l) | | | Root Length (mm) | | | | |
| 0 | 90.5 | 64.5 | 80 | 67 | 59 | 60.5 | 83 |
| .002 | 68 | | | | | | |
| .01 | | 46 | | | | | 22.5 |
| .02 | 51 | | | | | 47 | |
| .05 | | | | | | | 0.4 |
| .1 | | 54 | | | | | 0 |
| .2 | 32 | | | | | 14 | |
| 1.0 | | 39 | | | | | |
| 2.0 | 22 | | | | | 8 | |
| 10 | | 23 | | | | | |
| 20 | 0 | | | | | 0 | |
| 25 | | 8 | | | | | |
| 50 | | 0 | | | | | |
| 30000 | | | 74 | | | | |
| 50000 | | | 83 | | 37 | | |
| 70000 | | | | 75 | | | |
| 100000 | | | 70 | 60 | 30 | | |
| 150000 | | | | 38 | | | |
| 200000 | | | 36 | 32 | 14 | | |
| 300000 | | | | 0 | | | |
| 400000 | | | 0 | | 0 | | |

Example: Calculate by interpolation within each column an array of doses corresponding to an array of common percent responses, say EC50 at 50% response for Test No. 519: EC50 = 0.02 - [51-50(90.50)/100)(0.02 - 0.2)]/(51-32) = 0.075 mg/l. Enter in Row 50%, column No. 519 of Table 2.

The items in each column of Table 1 form a string of response values corresponding to matching doses. Repeated response values for a fixed dose in the data base were averaged to produce a single dose-response pair in the table. Each column defines a test. The tests highlight certain problems known to be common to bioassays: (1) Organisms react to chemicals with different sensitivities. The range of this difference when measured in relative dose levels is extremely wide, covering many powers of 10, for example, 6 in Table 1. (2) Test results differ widely between laboratories (519, 536), (931, 939) and within a laboratory (543, 544). (3) An enhanced response relative to a test control is often observed at low dose levels (543, 544).

The data in Table 1 will be analyzed stepwise, and sample calculations detailing each step are given in the tables generated in the process in each step. For a first reading, and for readers interested in an overview, the workings of the numerical examples at the bottom of each table may be skipped.

Step 1: Calculation of Coordinates of Centroid

Following the notation in Figure 1, calculate the pivots DP and RP on the dose and response axes respectively, one pair for each column in Table 1. Let D0, D1, ..., Dn denote the dose values (mg/l) and R0, R1, ..., Rn the corresponding response values (mm) for a test as shown in a single column. Let also A1, A2, ..., An denote the areas of a trapezoidal segment in a dose response curve calculated from A1 = 0.5(R0+R1)(D1-D0), A2 = 0.5(R1+R2)(D2-D1), ..., etc. The total area under the curve is A = A1 + A2+ ... + An. Then by definition of centroid

$$DP = [0.5(D0+D1)A1 + 0.5(D1+D2)A2 + , ..., ]/A.$$

$$RP = 0.5[0.5(R0+R1)A1 + 0.5(R1+R2)A2 + , ...,]/A.$$

The numerical value of RP is always smaller than the midvalue between the smallest and the largest of responses. Note also that the choice of trapezoidal rule for integration is arbitrary but reasonable. The numerical values of DP and RP corresponding to all tests are listed in Tables 3 and 4, respectively.

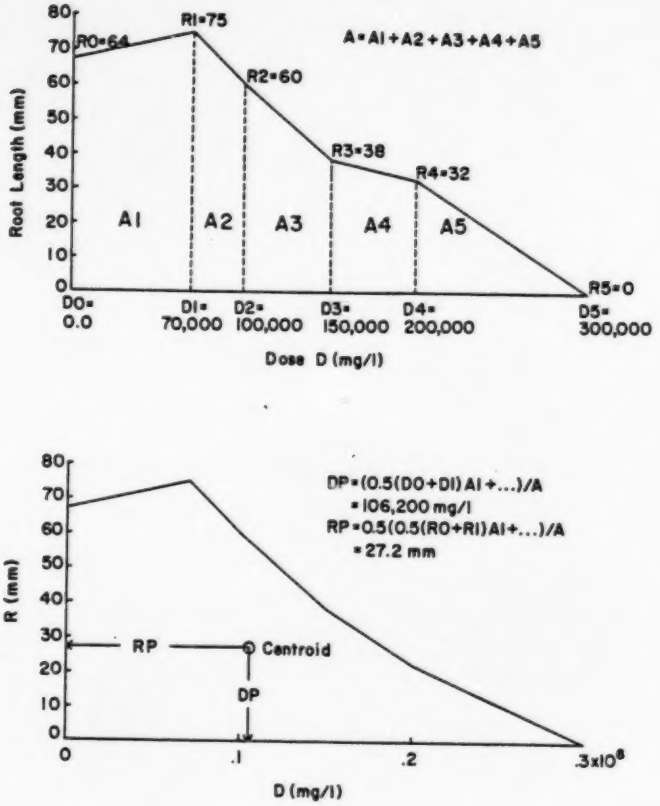


Figure 1: Calculation of centroid coordinates DP and RP for test No. 544.

Step 2: Interpolation for Dose Values Relative to a Common Response

This step facilitates capturing experimental variability in each test by interpolating the data at predesignated response intervals.

Convert response values, from root length (mm) to percents of root length at zero dose. Then, by interpolation, calculate dose values (mg/l) corresponding to an array of fixed response values (e.g., 0.1%, 0.5%, ..., 90%, 100%, 105%, ...) to all tests. The application of this step to the test data in Table 1 produces Table 2. Note the shift from dose to response entries from the body of Table 1 to the left column in Table 2 and vice versa. The first column in

Table 2. Typical examples of root bioassays showing dose for a common response in percent of control, interpolate from data in Table 1.

| | Wheat/2,4-D | | Wheat/Carbowax | | | Radish/2,4-D | |
|---|---|---|---|---|---|---|---|
| Lab No. | Lab 1 | Lab 6 | Lab 1 | Lab 2 | | Lab 4 | Lab 5 |
| Test No. | 519 | 536 | 543 | 544 | 548 | 931 | 939 |
| (R/R0)100 | | | D (mg/l) | | | | |
| .1 | 19.93 | 49.80 | 399556 | 299791 | 399157 | 19.86 | .099 |
| .5 | 19.63 | 48.99 | 397778 | 298953 | 395786 | 19.32 | .095 |
| 2 | 18.52 | 45.97 | 391111 | 295813 | 383143 | 17.28 | .079 |
| 5 | 16.30 | 39.92 | 377778 | 289531 | 357857 | 13.19 | .0497 |
| 10 | 12.60 | 29.84 | 355556 | 279063 | 315714 | 6.39 | .0407 |
| 20 | 5.19 | 20.10 | 311111 | 258125 | 231429 | .77 | .0228 |
| 30 | 1.07 | 13.65 | 266667 | 237188 | 176875 | .18 | .0096 |
| 40 | .16 | 8.43 | 222222 | 216250 | 140000 | .14 | .0082 |
| 50 | .075 | 4.80 | 188235 | 187500 | 103125 | .11 | .0069 |
| 60 | .0165 | 1.17 | 164706 | 145000 | 61429 | .078 | .0055 |
| 70 | .0069 | .63 | 141177 | 129773 | 40227 | .045 | .0041 |
| 80 | .0016 | .007,.073,.244 | 117644 | 114546 | 26818 | .018 | .0027 |
| 90 | .0008 | .0055 | 92307 | 99400 | 13409 | .009 | .0014 |
| 100 | | 0 | 0,43333,61538 | 0,86000 | 0 | 0 | 0 |
| 105 | | | | 29313,78300 | | | |
| 115 | | | | | | | |

Example: Convert dose values from mg/l to multiples of test DP. Use DPs shown on top of test columns in Table 3. For test No. 519 at 50% value of dose we have 0.075 (mg/l)/8.74 (mg/l) = 0.009 (dimensionless). Enter in Table 3.

Table 2 is the said array of percent responses selected to reproduce the original data at intervals not so coarse as to smooth out experimental fluctuations in the original data, yet not so fine as to add unnecessary detail and labor. Percentages greater than 100 are needed to recover enhanced response near zero dose. Multiple entries in a column reflect local fluctuations.

Figure 2a shows a plot of the original data and the interpolated dose for the array of % response (Step 2). The apparant smoothing between 0 - 40,000 mg/l is the consequence of selecting rather coarse array points between 90% and 110%. There is also slight smoothing at 200,000 mg/l. A finer spacing between

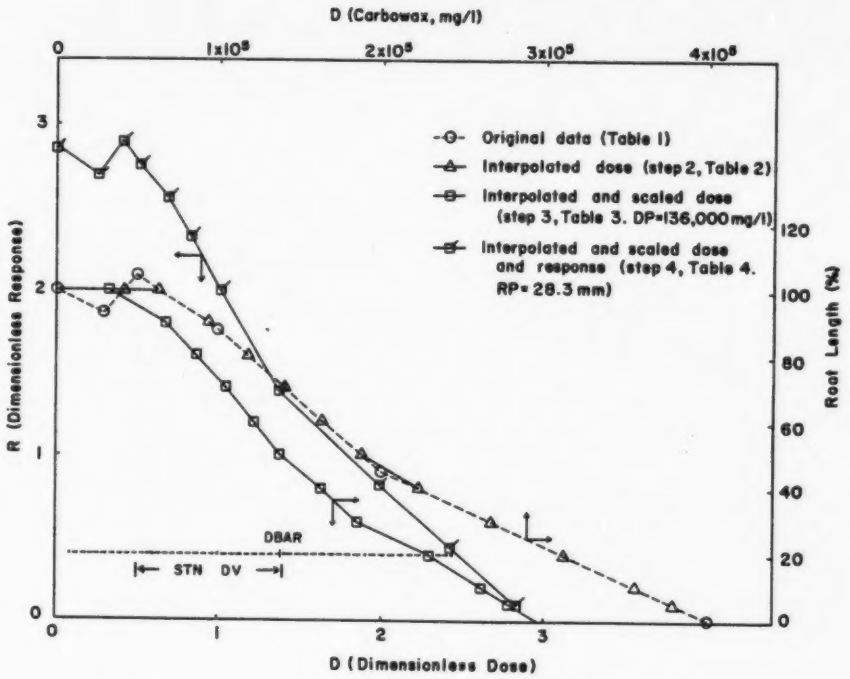


Figure 2a: A graphic display of scaling steps showing interpolation and scaling of test No. 543.

the points in the array would reduce or totally eliminate the smoothing effect. However, we will see that such level of resolution may not be warranted.

Step 3: Scaling The Dose Axis

In this step, the dose values of a test are standardized or normalized relative to the pivotal value of dose in that test and the variability relative to the dose axis is calculated.

Table 3. Typical example of root bioassay showing the role of DP as a scaler of dose data from Table 1. Dose in units of pivot (mg/l)/DP.

| | Wheat/2,4-D | | Wheat/Carbowax | | Radish/2,4-D | | Pooled Dose | | |
|---|---|---|---|---|---|---|---|---|---|
| Test No. | 519 | 536 | 543 | 544 | 548 | 931 | 939 | | |
| DP mg/l | 8.74 | 14.24 | 136063 | 106180 | 121905 | 8.28 | .0225 | | |
| (R/RO)100 | | | | D (mg/l)/DP | | | | DBAR | STD |
| .1 | 2.28 | 3.50 | 2.94 | 2.82 | 3.27 | 2.40 | 4.40 | 3.09 | .72 |
| .5 | 2.25 | 3.44 | 2.92 | 2.82 | 3.25 | 2.33 | 4.21 | 3.03 | .68 |
| 2 | 2.12 | 3.23 | 2.88 | 2.79 | 3.14 | 2.09 | 3.52 | 2.82 | .55 |
| 5 | 1.86 | 2.80 | 2.78 | 2.73 | 2.94 | 1.59 | 2.21 | 2.42 | .53 |
| 10 | 1.44 | 2.10 | 2.61 | 2.63 | 2.59 | .77 | 1.81 | 1.99 | .71 |
| 20 | .59 | 1.40 | 2.29 | 2.43 | 1.90 | .093 | 1.01 | 1.39 | .88 |
| 30 | .12 | .96 | 1.85 | 2.23 | 1.45 | .021 | .43 | 1.01 | .87 |
| 40 | .018 | .59 | 1.63 | 2.04 | 1.15 | .017 | .37 | .83 | .80 |
| 50 | .009 | .34 | 1.38 | 1.77 | .85 | .013 | .31 | .67 | .69 |
| 60 | .002 | .082 | 1.21 | 1.37 | .50 | .009 | .24 | .49 | .58 |
| 70 | .001 | .044 | 1.04 | 1.22 | .33 | .005 | .18 | .40 | .52 |
| 80 | 0 | 0,.005,.017 | .87 | 1.08 | .22 | .002 | .12 | .26 | .42 |
| 90 | 0 | 0 | .68 | .94 | .11 | .001 | .061 | .26 | .39 |
| 100 | 0 | 0 | 0,.32,.45 | 0,.81 | 0 | 0 | 0 | .16 | .28 |
| 105 | | | | .28,.74 | | | | .51 | .33 |
| 115 | | | | | | | | | |

Example: (a) calculate RBAR at 50%: (0.009+0.34+...)/7=0.67

(b) use 0.67 as a common dose and interpolate in column No. 519, find the response in %=10-[(10-20)(1.44-.67)/(1.44-.59)]=19.1%

(c) Convert 19.1% for Test No. 519 from % of control (i.e.,90.5 mm, Table 1) to multiples of RP (7.59 mm, Table 4) ((19.1)(90.5)/(100(7.59))=2.28. Enter in Table 4 column 519 across 0.67.

Divide the dose columns in Table 2 by corresponding dose pivot DP of that test to produce Table 3. This simple normalization rearranges the dose values of all tests so that they are better matched relative to one another at a common percent response. The realignment unifies a wide range of dose levels (zero to 400,000 mg/l) by placing the data in a narrow band (zero to 4.4), expressed not in the original units of mg/l but in units (or multiples) of test DPs. That is, in terms of a scaled or dimensionless dose. As a result of this unification, it becomes more meaningful to seek a common dose by pooling or averaging the values along a row in the table to obtain the last two columns,

i.e., the mean, DBAR, and the standard deviation. DBAR, which is a dimensionless average dose is one coordinate of a unified curve. The second coordinate, RBAR, will be calculated shortly.

The scaling of the dose is shown plotted in Figure 2a (Step 3). Since the process involves a single linear transformation, there is a shift of the scaled curve to the left of the original. The averaging of the scaled dose at one of the common % response points in the array (20%) is shown in Fig 2a. The example demonstrates calculation of DBAR (= 1.39) and standard deviation (0.88) of seven scaled dose points as in Table 3. The full data for test 534 are also shown for comparison. Since the sole purpose of selecting a percent array is to define a scaled common dose, that is, to obtain DBAR, the % array points need not be too closely spaced.

Table 4. Typical examples of root bioassay showing the role of RP as a scaler on data from Tables 1 and 2.

| | Wheat/2,4-D | | Wheat/Carbowax | | Radish/2,4-D | | | Pooled R | |
|---|---|---|---|---|---|---|---|---|---|
| Test | 519 | 536 | 543 | 544 | 548 | 931 | 939 | | |
| RP mm | 7.59 | | 11.78 | 28.29 | 27.22 | | 14.95 | 3.73 | 15.14 |
| DBAR (mg/l)/DP | Scaled Root length (R/RP) | | | | | | | RBAR | STD |
| 3.09 | | | | .17 | .11 | .16 | | .15 | .03 |
| 3.03 | | | | .19 | .14 | .17 | | .17 | .03 |
| 2.82 | | .28 | .10 | .003 | .26 | .19 | .17 | .12 | |
| 2.42 | | .44 | .45 | .51 | .49 | .0002 | .24 | .36 | .20 |
| 1.99 | .42 | .61 | .82 | 1.04 | .74 | .42 | .42 | .64 | .24 |
| 1.39 | 1.26 | 1.17 | 1.40 | 1.44 | 1.26 | 1.02 | .84 | 1.20 | .21 |
| 1.01 | 1.78 | 1.63 | 2.00 | 1.06 | 1.74 | 1.38 | 1.09 | 1.67 | .34 |
| .83 | 2.05 | 1.88 | 2.32 | 2.42 | 1.99 | 1.57 | 1.22 | 1.92 | .42 |
| .67 | 2.28 | 2.12 | 2.56 | 2.74 | 2.18 | 1.73 | 1.33 | 2.13 | .48 |
| .49 | 2.53 | 2.52 | 2.78 | 2.68 | 2.39 | 1.91 | 1.46 | 2.47 | .31 |
| .40 | 2.56 | 2.71 | 2.89 | 2.64 | 2.50 | 1.99 | 1.86 | 2.46 | .39 |
| .26 | 2.86 | 3.04 | 2.69 | 2.58 | 3.03 | 2.14 | 3.18 | 2.79 | .35 |
| .26 | 2.86 | 3.04 | 2.69 | 2.58 | 3.03 | 2.14 | 3.19 | 2.79 | .36 |
| .16 | 3.35 | 3.26 | 2.68 | 2.53 | 3.38 | 2.77 | 4.06 | 3.15 | .53 |
| .51 | 2.50 | 2.47 | 2.75 | 2.69 | 2.36 | 1.89 | 1.44 | 2.30 | .47 |

Example: Calculate the common response RBAR corresponding to DBAR of 0.67: [2.28+2.12+2.56+2.74+2.18+1.73+1.33]/7=2.13. The point (0.67,2.13) defines one coordinate on a unified dose response curve of seven tests in Table 1.

Step 4: Scaling The Response Axis

In this step, the response values of a test are standardized relative to the pivotal value of response in that test and the variability relative to the response axis is calculated.

The transformation may now be completed by rearranging the response values so that they, too, are better matched with one another relative to a common dose. The common dose to be used is the scaled mean dose DBAR just calculated in Step 3. For each value of DBAR we interpolate in the dose columns of Table 3 to obtain a matching percent response. When DBAR is greater than the dose values in a column, no entry is made, that is no extrapolation is carried out. The percent response from each test is transformed by multiplying it by (control (mm))/(100 RP(mm)) and an entry is made in Table 4. Notice again the reversal of dose from the body of Table 3 to the left column in Table 4 and vice versa.

Having already used the percent response as an intermediate step in the overall transformation, we now will deviate from the current practice which retains that expression as a common response. In its place, we use the scaled mean response RBAR. The latter is obtained by averaging all responses to a common DBAR. The process is graphically displayed in Fig. 2b. The experimental variability along the response axis is listed in the last column of Table 4.

Step 5: Unified Curve of Example Data

Finally, we pair up the overall scaled dose DBAR with the response RBAR from Table 4 to be used as a unified relationship representing the data in Table 1. Hereafter, we denote DBAR and RBAR simply by D and R. Since we will be dealing with dimensional and dimensionless quantities, we follow the mention of all dimensional quantities with appropriate units, for example, mm, mg/l, ... etc. This would remove the confusion in identifying the dimensionless quantities which in this report are normalized by DP or RP.

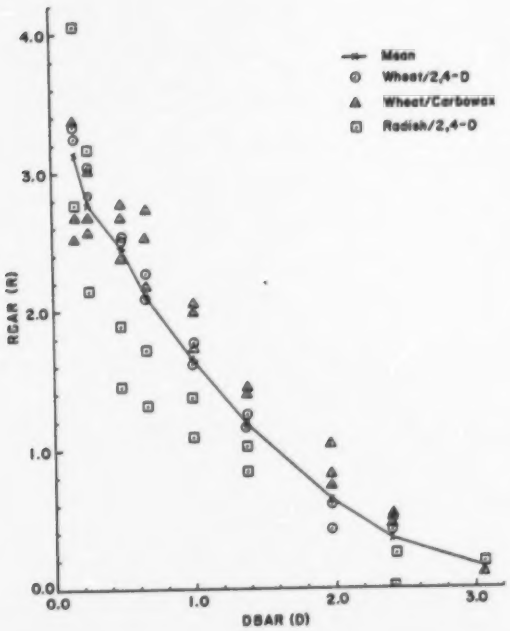


Figure 2b: A graphic display of a scaling step showing the merging together of points from various tests and averaging along the response axis to form a unified curve.

The relationship between the unified, i.e., the scaled dose response (D, R) curve and the individual root and chemical data are shown in Fig. 2b. It reveals the role of the scalers in retaining the experimental variability inherent in the original data. The merging and relative placements of data points from various tests along the vertical axis is apparent. Wheat/2,4-D data are approximately centrally located along the vertical axis for all values of the dose whereas wheat/Carbowax data are on the top at large values of the dose and drop below at small values; radish/2,4-D data display an opposite trend. These follow from applying Steps 3 and 4.

A listing similar to Table 4 constructed for Ratsch's entire data base would contain several thousand entries. It is not useful to show a table so extensive nor would the display of thousands of points in a single graph be greatly insightful. A graphic display of grouped data is more useful and practical. The groupings are relative to single root and single chemical. The above steps are applied to all tests belonging to a group.

## A Unified Curve of Whole Data Set

Table 5 summarizes groupings of 560 tests selected from an original data base of more than 1000 (Ratsch 1983). The entries in the table and the selection procedure are discussed below. Results of applying the scaling procedure to these data are shown in Figure 3. Each point represents a scaled dose (DBAR), or simply D, and scaled response (RBAR), or simply R, along a dose-response curve of a group in Table 5. The solid curve is the unified relationship for all data. Other curves will be explained later. Evaluation of the statistical variability around the unified curve (solid line) is based on individual data points and not merely their group representation.

## Preliminary Error Analysis

The statistical representation of experimental variability can be displayed exactly (Figure 4) by plotting the local standard deviation along the dose and the response axes, respectively. These curves can be interpreted most conveniently by studying their meanings in conjunction with the example calculations in Table 3 (for dose) and Table 4 (for response) or for the point in Figure 2b.

The numbers of data points are relatively sparse at low values of response and at high values of dose. This is a reflection of missing data points at high dose values. They are equally abundant in the midrange. For this reason, we recommend that use of the unified relationship be within approximately 0.7 and 1.8 for both D and R. Numbers on the dose curve that are greater than 560

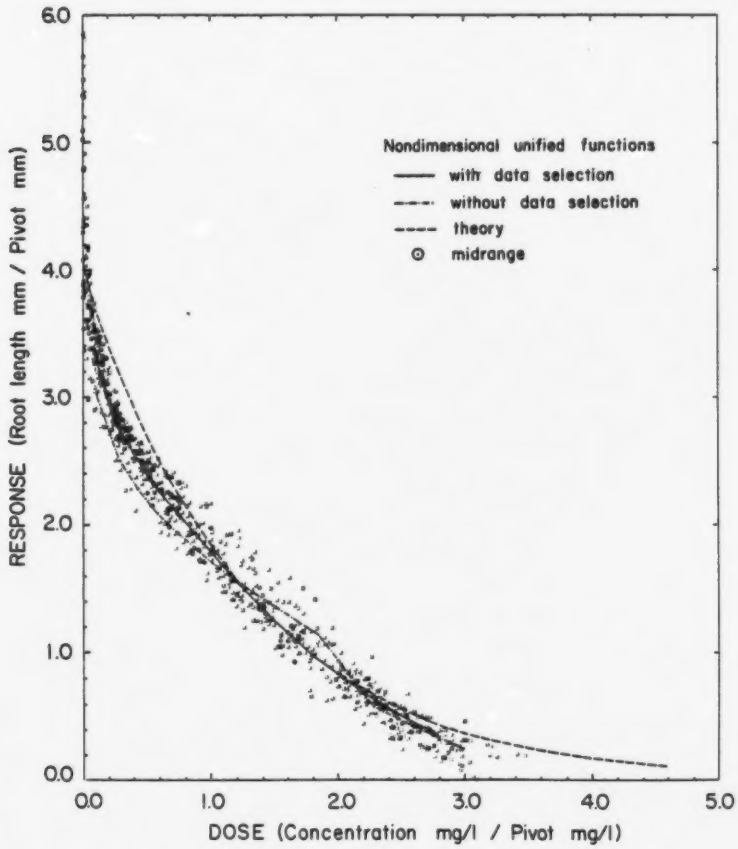


Figure 3: A unified dose-response pattern emerging from scaling root elongation bioassay tests, and comparison of theory and unified functions with and without data selection.

indicate multiple values at those points, reflecting the local fluctuation of the original data points.

Table 5. Summary of characteristics of Root Bioassay data base listing coordinates of group centroid (DP and RP), the sensitivity factor k( RM/S ), number of tests in each group, and a code referring to the root and chemical compound.

| | Ag NO3 (1) | NaF (2) | CdCl2 (3) | MAA (4) | 2,4-D (5) | Carbowax (6) | Monuron (7) | Cineole (8) | All chem. |
|---|---|---|---|---|---|---|---|---|---|
| Red Clover (1) | | | | | | | | | |
| DP mg/l | 182 | 265 | 61.3 | 382 | .596 | 105000 | 185 | 978 | 13200 |
| RP mm | 7.06 | 9.16 | 7.05 | 4.50 | 4.88 | 9.84 | 6.94 | 8.59 | 7.31 |
| k | 1.04 | 1.17 | 1.18 | 1.45 | 1.41 | 1.39 | 1.24 | 1.38 | 1.25 |
| Test No. | 1-14 | 15-37 | 38-58 | 59-72 | 73-89 | 90-104 | 105-112 | 113-121 | 1-121 |
| code | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | R1 |
| Lettuce (2) | | | | | | | | | |
| DP mg/l | 43.3 | 322 | 23.4 | 186 | .181 | 96800 | 151 | 655 | 13700 |
| RP mm | 10.7 | 22.5 | 13.9 | 10.8 | 13.4 | 21.5 | 18.0 | 15.6 | 16.1 |
| k | .991 | 1.32 | .940 | 1.49 | .999 | 1.51 | 1.52 | 1.12 | 1.19 |
| Test No. | 122-134 | 135-151 | 152-168 | 169-179 | 180-194 | 195-210 | 211-222 | 223-236 | 122-236 |
| code | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | R2 |
| Wheat (3) | | | | | | | | | |
| DP mg/l | 296 | 298 | 287 | 816 | 14.0 | 110000 | 295 | 743 | 18900 |
| RP mm | 17.4 | 20.5 | 15.5 | 21.0 | 11.9 | 27.8 | 24.7 | 21.4 | 20.6 |
| k | 1.38 | 1.28 | 1.58 | 1.54 | 1.46 | 1.27 | 1.41 | 1.20 | 1.33 |
| Test No. | 237-242 | 243-260 | 261-269 | 270-278 | 279-285 | 286-299 | 300-301 | 302-319 | 237-319 |
| code | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | R3 |
| Cucumber (4) | | | | | | | | | |
| DP mg/l | 164 | 253 | 87.4 | 129 | 1.11 | 105000 | 170 | 958 | 13800 |
| RP mm | 22.4 | 28.5 | 24.2 | 29.3 | 19.2 | 29.3 | 320 | 4.4 | 26.6 |
| k | .957 | 1.25 | 1.03 | 1.36 | 1.35 | 1.69 | 1.78 | 1.23 | 1.27 |
| Test No. | 320-333 | 334-357 | 358-374 | 375-386 | 387-406 | 407-422 | 423-432 | 433-442 | 320-442 |
| code | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | R4 |
| Radish (5) | | | | | | | | | |
| DP mg/l | 208 | 262 | 75.1 | 128 | .879 | 105000 | 152 | 845 | 11700 |
| RP mm | 27.6 | 48.5 | 29.5 | 28.4 | 26.7 | 41.9 | 42.6 | 41.9 | 36.7 |
| k | 1.48 | .979 | 1.18 | 1.29 | .933 | 1.26 | 1.49 | 1.11 | 1.17 |
| ~~No.of Tests~~ | 443-458 | 459-480 | 481-496 | 497-508 | 509-520 | 521-533 | 543-548 | 549-560 | 443-560 |
| code | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | R5 |
| All Roots (6) | | | | | | | | | |
| DP mg/l | 167 | 276 | 86.9 | 307 | 2.02 | 104000 | 167 | 810 | 14000 |
| RP mm | 17.4 | 26.1 | 17.6 | 18.3 | 15.1 | 25.6 | 27.2 | 24.2 | 21.5 |
| k | 1.12 | 1.17 | 1.12 | 1.42 | 1.19 | 1.41 | 1.50 | 1.19 | 1.23 |
| ~~Test No.~~ | 63 | 104 | 80 | 58 | 71 | 74 | 47 | 63 | 560 |
| code | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | RC |

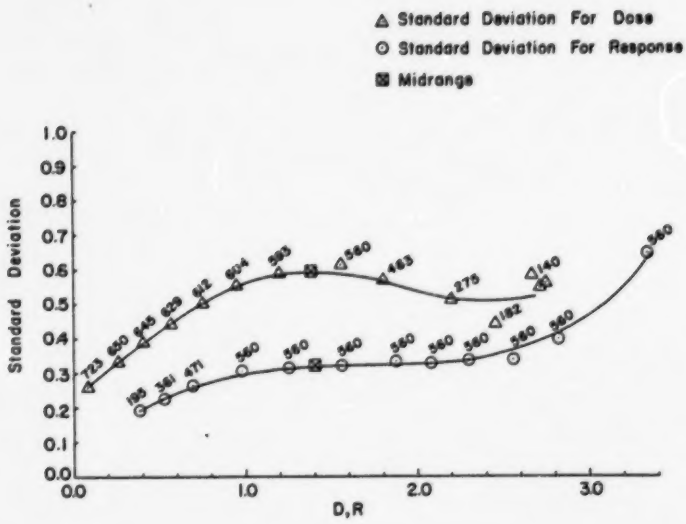


Figure 4: An analysis of experimental variability along the axes of the unified function,

## Selection Procedure

The purpose of test selection is to reduce the inconsistancy resulting from the use of incomplete tests in producing the dose response curve and to base a unified function on well-defined dose-response relationships. A test conducted at one or two dose levels or at several dose levels all close to zero does not define a dose-response curve at all. Establishing whether or not a test does provide a well-defined dose-response relationship is complicated by the presence of experimental error in the data. The decision criterion explained here uses information generated in the scaling process and fits our procedure well. Any other reasonable criterion may amend or replace ours if desired.

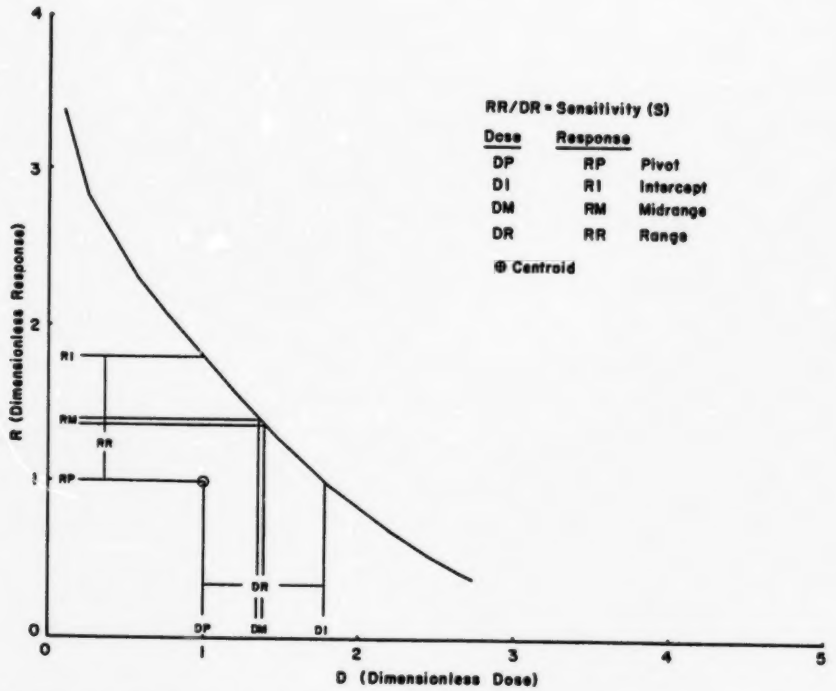


Figure 5: Definition of scalers.

To explain the selection procedure, we introduce parameters we consider important for better understanding of the overall scaling approach. Figure 5 provides a graphic presentation of the definitions of DP and RP. If we draw two lines parallel to each axes through the centroid to intercept the dose-response curve, we obtain the dose intercept DI and the response intercept RI as shown in Figure 5. RR and DR are the ranges along the response and dose axes, respectively. They are obtained from RR = RI-RP, and DR = DI-DP. RM and DM are points located approximately midrange within RR and DR segments, respectively. The slope S is calculated at midrange and approximated by S = RR/DR.

When the dose and response coordinates are dimensional, the slope has the value of sensitivity expressed in (mm)/(mg/l). In a dimensionless system it has the same interpretation but must be converted to dimensional values by multiplying it by the ratio RP/DP. Ideally, the slope at midrange is obtained by differentiation. But the stated approximation is more meaningful for wildly fluctuating and sparse data. The slope S will be referred to as the SENSITIVITY. It is evaluated for every test and for the scaled and unified group curves.

Figure 5 defines the range, the midrange point and the sensitivity of an organism to a chemical along the central portion of the dose-response relationship. These are quantities that can be useful in describing a test. Now, we shall use these notations to describe our selection criterion.

When a test is terminated at a dose value smaller than DI, an intercept, a range, and a sensitivity for that test cannot be calculated. This probably would not present a problem except that it is our intent to establish a complete dose-response trend based on existing data. For this reason, we used the existence of DI as a criterion for retaining a test while synthesizing the data base.

In spite of the need to select a complete test, if we apply no restriction at all to the use of a test and let all tests with two or more data points make their contributions to calculation of the unified relationship, we obtain a slightly modified function as shown by the dot-dashed curve in Figure 3. The function is identified by the inflection point on its central portion. The presence of an inflection point can be attributed exactly to the use of an excessive number of tests in a data base having a few data points near zero. Mathematically, this happens as a result of overestimating the values of RP, nd underestimating DP in those tests.

## DISCUSSION AND GUIDELINES FOR USE

Up to this point the scaling process has been presented as a formal mathematical procedure to illustrate its effectiveness in organizing and unifying diverse data. Our objective now is to develop a practical and theoretical working method for prediction and experimental design relative to this data base or relative to the use of the method for other data bases.

### Theoretical Analysis

Returning to Figure 3, we attempt to generalize the unified mean curve by comparing it with a logarithmic function $D = a + b.k.\ln(R/Ro)$ where a, b and k are constants. The function can be made to satisfy the condition that at zero dose, the response equals the control value (i.e., at $D = 0, R = Ro$) simply by setting ($a = 0$). Furthermore, the condition that the slope of the dose-response curve at midrange equals to sensitivity S can be satisfied by differentiation of the function, evaluation at midrange and substitution. This gives $b = -1$ and $k = RM/S$. By satisfying these two conditions, we have assigned particular meanings to the constants of the function which are purely theoretical. Their real values can be found from fitting the logarithmic function to groups of data in Table 5.

The means (and the standard deviations) of 54 sets of constants a, b and k representing the groups in Table 5 are: $a = -0.04$ (and 0.28), $b = -1.06$ (and 0.24), and for $k = 1.28$ (and 0.19). In spite of the fact that the variability is great, the mean values of a, b, and k are close to the theoretical values and the coefficients of correlation for the fit in all cases are greater than 0.95. Consequently, the theoretical function may be good for prediction. We refer to k as SENSITIVITY CONSTANT. It is distinguished from sensitivity S in that it is calculated from it and introduced to satisfy boundary condition of the problem.

We have examined many functions that fit the unified curve rather well. However, none would so readily satisfy the initial and boundary conditions as would the logarithmic function. The satisfying of these conditions enables simple interpretation of the parameters of the functions a, b and k in terms of biological components of the problem. This can be an important theoretical consideration. It means that we can anticipate the form of the unified function by direct calculation of its parameters from the biological data. The equation can be written in several convenient ways:

$$D = -k \ln(R/Ro) \qquad (1)$$

$$R/RO = \exp[\ -D/k\ ] \qquad (2)$$

$$d(R/Ro) \ / \ dD = -\ k(R/Ro) \qquad (3)$$

where the sensitivity constant $k = RM/S = 1.28$ for the data base as a whole. The plot of equation (1) is shown as a dashed curve in Figure 3.

The above results, of course, would be valid for the data used in this analysis. It may also be useful for extension to similar data if ones used in this report are considered representative. Beyond that, however, the procedure itself can be potentially useful as a general means of organizing and using large and diverse data sets.

## Practical Applications

For the dimensionless unified curve and the associated equations to be of direct use in determining toxicity, they must be used together with information on mean sensitivity of crop roots to chemicals. Since the equations and the unified curve are nondimensionalized with respect to pivots, the mean values of dose and response pivots for a group of tests using one crop root and one chemical compound is sufficient to construct parts or complete mean dose response curve of those tests. We will not undertake this task ourselves but some readers might have enough special interest in the particular data we used to benefit from undertaking it themselves. The following 3 paragraphs summarize the most important results applicable to prediction, error analysis, and experimental design.

Of an important practical value is that the dimensionless midrange is nearly constant, approximately equal to 1.4 for all groups of tests. When multiplied by the mean dose pivot of a group, it will yield an effective and convenient scale for summarizing the toxicity of the chemical tested in that group.

If, on the other hand, we conduct a single test and wish to relate it to the database, we can in a like manner, compare the entire dose response or merely the midrange and the EC50 of the test with the unified curve, thereby determining the experimental error associated with one test.

Finally, if we wish to design new experiments, we can use the equations for predetermining the dose intervals best suited to the tests. We shall discuss these aspects of the application in some detail below, starting first with general results.

Table 5 contains the pivot values DP and RP. These can be interpreted as weighted average values of dose and response. Their numerical values are always less than the midrange values. Since the overall average of the dimensionless midrange equals 1.4, dose pivots can be converted by multiplication to numerical toxicity indices for various chemicals. That is, the midrange can be used to rank the sensitivity of crop roots to the chemicals. The plot of ranked DP's are shown in Fig. 6.

The roots are also ranked according to their controls in that figure. The data points are marked to identify them with the entries in Table 5. The variability of the data are marked for each group indicating considerable overlap of DP values among chemicals. The figure provides a useful way to rank tested chemicals and the potential to predict general effects of untested chemicals known to fall within a similar class. The figure can also be used to pick approximate DPs for use in converting scaled nondimensional dose to mg/l.

Other potential uses and guidelines are:

(1) The nondimensional values of the commonly used EC50 and the midrange are provided for the data base as a whole: Midrange values are RM = DM = 1.4

(Figures 3, 4 and 5), and the overall data base EC50 can be found from equation 1 by expressing R/Ro as a decimal. Thus D = -1.28 ln(0.5) = 0.88.

The experimental error for any point on the unified curve can be found in a general way from Figure 4. For example, the standard deviations at midrange and EC50 are 0.59 and 0.54, respectively. The dimensionless midrange, EC50,

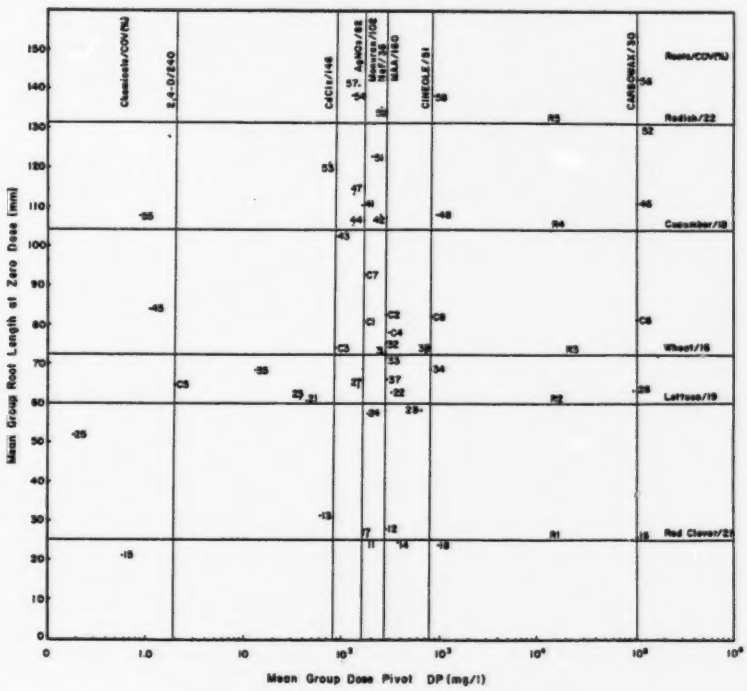


Figure 6: Ranking of grouped bioassay tests relative to mean root length at control and mean dose pivot DP. Grouping codes in the graph are identified in Table 5. COV is the coefficient of variation (%) using all tests in a group.

and the associated standard deviations of the errors can be converted to toxicity values by multiplication by an appropriate dose pivot.

Generalized values of dose pivots in mg/l for chemicals used in the data base can be obtained from Figure 6 and Table 5. For example, DP = 2.02 mg/l for 2,4-D, for all roots, 1.11 mg/l for 2,4-D/cucumber, ..., etc. The variability is considerable as can be seen from the coefficient of variations in Figure 6.

(2) The total distributions of the midrange and EC50 for the data base as a whole are displayed in Figure 7. The arithmatic mean of the midrange is, as expected, equal to 1.4. The sharp, nearly step-function distribution of the midrange is an indication of the effectiveness of DM as an end point scaler of the problem. Its value falls within safe values of the data range. In contrast, the distribution of EC50 is by comparison gradual, bimodal and covers a wide range of values. The measured arithmatic mean for the data base is 0.95, well within the error terms of theoretical value of 0.88.

The most probable value of EC50 seldom (probability less than 1%) coincides with a midrange value and the probability that EC50 is numerically less than a midrange value equals 82%. The most probable value of midrange according to Equation 2 is at $R/Ro = \exp(-1.4/1.28) = 0.34$ or 34%. Therefore, to the extent that EC50 is intended as an end point scaler to express a central tendency of the data, its use in comparison with midrange is less fulfilling.

(3) There will always be differences in values of EC50 or midrange when comparing generalized results with a particular test. If a test happens to be a part of the data base, the comparison of the two values gives an idea of the relative precision of that test in representing the chemical effect for the group or the data base as a whole. If it is a new test with one of the roots and chemicals of Table 5, then the comparison provides a link with the present

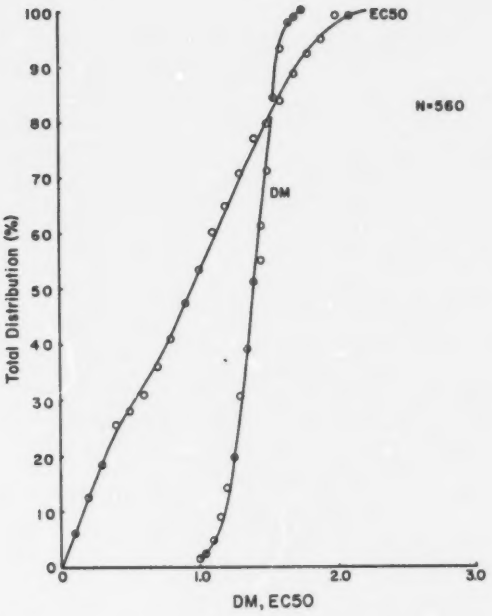


Figure 7: Distribution of dimensionless EC50 and midrange (DM) for root elongation data base. The step-like distribution of DM demonstrates its effectiveness as a scaler to be used in summarizing bioassay results.

data base. In all situations, Figure 4 provides a convenient way to estimate the relative precision of a test for all values of dimensionless D and R. For example, from Table 3 the EC50 for test 548 is 0.85 and its standard deviation from Figure 3 is 0.56. The EC50 for Test 931 is 0.013, a value that is too close to the origin and according to our analysis is anomolous. A more likely value of EC50 according to our analysis may be obtained from Equation 1 by using the value of the sensitivity constant in group 55, i.e., radish/2,4-D ($k = 0.933$). Thus, $EC50 = D = -0.933 \ln(0.5) = 0.64$ with a standard deviation from Figure 4 is equal to 0.45.

(4) When designing an experiment, it is useful to know in advance the dose levels to be used with a chemical and an organism. The analysis presented in this paper provides a simple treatment of the problem. For example, Figure 6 may be consulted to obtain an estimate of a pivot for the subject chemical. Then, Equation 1 can be used to obtain an appropriate spacing of dose levels to be applied. For example, it may be desired to apply dose levels near EC90, EC30, and EC10. The scaled dose for EC90 is $D = -1.28 \ln(0.9) = (-1.28)(-0.105) = 0.14$ and for EC30 and EC10 are 1.54 and 2.95, respectively. Suppose now that we want to test silver or cadmium compounds where DP = 100 mg/l (Figure 6). The dose levels to be tested therefore are 14, 154, 295 mg/l, respectively. Once a test is conducted, a new DP can be calculated from the test results and midrange and other EC values obtained directly from the data.

(5) It is useful to compare our results with the EC50 calculations of Ratsch. Ratsch applied a selection procedure to the data somewhat different from ours. Our criterion was an intercept at high dose values. He plotted the test data on a logarithmic paper, selected the nearly linear portion and omitted all data falling outside 20-80% of root length reduction relative to control. The most probable value of an intercept in our analysis corresponds to 28% root reduction with a standard deviation of 0.56. Thus, in comparison with Ratsch, we have been less selective and once a test was picked, little smoothing and no linearization or data omission was done. Additionally, by comparison of distributions in Figure 3, we have shown the consequence of omitting incomplete tests. Ratsch reported his results in geometric means which we converted into arithmatic means for comparison with our data. The distributions of EC50 for two methods of calculation are compared in Figure 8. There is a slight difference in the overall mean, 0.6 for Ratsch and 0.95 for ours, but the trend is similar. Considering the differences in approach and that the standard deviation at D = 0.95 is 0.55, the agreement is good.

(6) Finally, we must emphasize the simplicity of using our results, be it the equations or simply the overall mean values of midrange and EC50 together with values of DP obtained from Figure 6 or Table 5. It would seldom be necessary to follow the entire five steps shown in the procedure section. Perhaps the calculation of the pivot would be needed when comparing a single test with our results. However, the entire procedure must be followed step by step if new data were to be added to the existing data base. That situation requires computer use. The authors would be pleased to share their programs with any potential user.

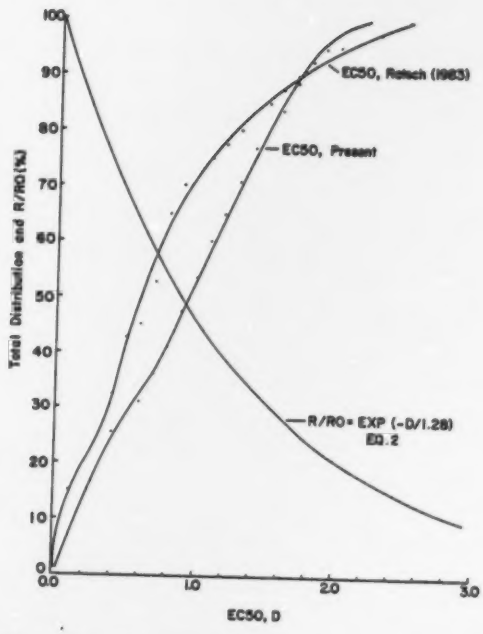


Figure 8: EC50 distribution comparing our calculation with the linearized approach of Ratsch.

## CONCLUSION AND RECOMMENDATION

The coordinates (DP,RP) of a centroid obtained from a bioassay test can be used to (1) unify a diverse data set, (2) provide a procedure for systematic addition of new data, (3) enable a theoretical formulation that appears simple and useful, 4) facilitate the design of new experiments, the prediction of general effects, and the evaluation of experimental error.

The numerical values of midrange, sensitivity constant, etc., are particular to the data set used. Whether or not this data set is representative of all crop root bioassays or there may exist others that are, is an open question but separate from the potential utility of the approach itself.

The authors recommend the application of this approach to other bioassay problems with the added emphasis on the use of midrange as an end point scaler for summarizing a test.

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